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Laser Induced Bubble Formation in the Retina

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Background and Objective: **The immediate thermodynamic effects of absorption of a laser pulse in the retina are theoretically studied to understand underlying physical damage mechanisms at threshold fluences. Damage is most likely to occur at threshold levels in the retinal pigment epithelium due to the strong absorption by the melanosomes.**
Methods: **The retinal pigment epithelium is modeled as an aqueous environment with absorption occurring at small spherical sites with absorption coefficients representative of melanosomes. For laser pulse durations of less than 10^{-6} seconds, heat conduction is negligible during energy deposition and the resulting large energy density in the melanosomes will cause vaporization of the medium immediately surrounding a melanosome.**
Results: **We developed expressions for calculating the size of bubbles produced as a function of pulse characteristics and melanosome properties. We show that for pulse durations between 10^{-6} and 10^{-9} seconds, bubble formation will occur for laser fluences that are smaller than those required to cause Arrhenius-type thermal damage.**
Conclusion: **Bubble formation is likely to be the mechanism of threshold damage to the retina for laser pulses durations in the time regime between 10^{-6} and 10^{-9} seconds.**

Key words: Arrhenius, bubble, damage, ED_{50}, fluence, laser, minimal visible lesion, retina, thermal damage, threshold, vaporization

INTRODUCTION

Continual progress in laser development leads not only to more beneficial uses of lasers, but also to increased danger of injury to the visual system. The potential for injury, and the importance of understanding how it occurs, continues to grow as a result of the steady advancement in laser technology, which has provided increasing pulse energies and shorter pulse durations.

The theoretical research described here was undertaken in order to understand the primary effects of the laser energy immediately after absorption in the retina. The main absorption site for laser pulses is expected to be the melanosomes in the retinal pigment epithelium (RPE). Our model incorporates melanosomes as spherical absorption sites that are 1 micron (10^{-6} m) in radius and have absorption characteristics of melanin. To represent the surrounding cellular material, these melanosomes are embedded in a homogeneous nonabsorbing medium with the thermal characteristics of water.

It is well known that damage can occur in cells due to temperature rises. This thermal damage has been modeled [1–3] in terms of an Arrhenius-type activation process. However, it is also

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known that damage can occur on the cellular level due to bubble formation [4,5]. Significant heat conduction away from a melanin granule requires time scales on the order of microseconds. For pulses of shorter duration than this, most of the pulse energy remains localized at the absorbing melanosome during the pulse and temperature rises are expected to be high enough to cause vaporization of the immediate surrounding medium. This will create a bubble that then expands outward from the melanosome. In this paper, expressions are developed to calculate the maximum size expected for the expanding bubble as a function of the laser pulse parameters and properties of the melanosomes and surrounding cellular medium. Tables are included with a range of representative cases. We also show that for pulse durations between 10^{-6} and 10^{-9} seconds, damage due to bubble formation and growth will occur at fluences (J/cm^2) lower than those needed for Arrhenius thermal damage. This implies that the mechanism for threshold damage in this pulse duration regime is bubble formation.

Functional impairment to the visual system will occur if there is damage to photoreceptors or their associated nerve transmission pathways. This can occur as a secondary effect of damage that occurs initially in other cellular layers in the retinal region. In this paper we investigate damage due to bubble formation in the RPE. We refer the reader to Sliney [6] for discussions of other damage mechanisms that are not relevant to threshold damage for submicrosecond pulses.

An especially well-studied damage mechanism is that due to temperature rises, which lead to protein denaturation and subsequent cellular dysfunction through an Arrhenius thermally activated process [1–3]. For pulse lengths longer than 10^{-5} seconds, this thermal damage is likely to be the dominant mechanisms for threshold damage. However, we show that for pulse durations less than 10^{-6} seconds, damage from bubble formation will likely occur at lower fluences and therefore determines damage thresholds. Between 10^{-5} and 10^{-6} seconds there is a smooth transition from thermal damage to bubble damage as the threshold mechanism.

The most important site for retinal damage is the retinal pigment epithelium, which absorbs approximately 50% of incident visible radiation [7], an order of magnitude more energy than absorbed by the photoreceptors. This strong absorption by the melanosomes makes the RPE the likely location for the source of temperature rises and bubble formation that can lead to damage to the retina at threshold levels of irradiance [8,9]. Evidence that near-threshold damage is centered in the RPE was observed by Gueneau et al. [10]. Absorption by the choroid is less likely to be the site of threshold damage since it is located posterior to the RPE and thus receives less incident light than the RPE, and also has blood flow to help with heat removal.

METHODS

Analysis of Bubble Formation and Growth

In our model the absorption of light occurs in melanosomes, which are represented as spheres described by two parameters that can be varied: the radius R_a and the absorption coefficient α. These absorbing spheres are also given the following thermal characteristics: the specific heat of melanin of $c_m = 2.51$ J/g·°C and the density of melanin of $\rho_m = 1.35$ g/cm^3 [11]. These melanosomes are embedded in a surrounding cellular medium that has the thermal characteristics of water; heat capacity $c = 4.19 \times 10^3$ J/kg·°C, density $\rho = 10^3$ kg/m^3, and thermal conductivity $\kappa = 0.57$ J/m·s·°C.

Thermodynamic conditions of bubble growth. We now investigate bubble generation resulting from laser pulses with durations of less than a microsecond. The melanosomes are approximately 1 μm $= 10^{-6}$ m in radius, with bubbles developing around them, and the surrounding cellular material is treated as water. Using the thermal properties of water given earlier and $L = 1$ μm as a characteristic size for the system, we find that the approximate speed for heat conduction is on the order of

$$v(\text{thermal}) \approx \kappa/\rho cL \approx 0.1 \text{ m/s}. \quad (1)$$

During a laser pulse of $\tau_p < 10^{-6}$ seconds, heat conduction occurs over a negligible distance from the melanosome, and thus negligible heat loss occurs during absorption and subsequent bubble growth.

The adiabatic nature of the process allows for the calculation of the important parameters characterizing the growth of a bubble. Under adiabatic conditions, the relationship PV^γ = constant holds and therefore

$$\frac{V}{V_o} = \left(\frac{P_o}{P}\right)^{1/\gamma}. \quad (2)$$

In Equation (2), V_o and P_o are the volume and pressure of the bubble at the end of the laser pulse

but before the bubble starts its adiabatic expansion, and γ is the ratio of the specific heat of the vapor at constant pressure to the specific heat at constant volume. Equation (2) shows that the maximum radius attained by the bubble after expansion can be calculated if the initial radius of the bubble surrounding the melanosome at the end of the laser pulse can be determined. The radius of the melanosome itself will be denoted by a. The radius of the bubble and the pressure within will be denoted by r and P, and their values immediately after laser absorption will be r_o and P_o. The volume of the vapor at any time is therefore

$$V(r) = \frac{4\pi}{3}(r^3 - a^3). \tag{3}$$

Combined with Equation (2), this gives

$$r^3 = a^3 + (r_o{}^3 - a^3)\,(P_o/P)^{1/\gamma}, \tag{4}$$

which can be rewritten in terms of dimensionless quantities as

$$(r/a)^3 = 1 + [(r_o/a)^3 - 1](P_o/P)^{1/\gamma}. \tag{5}$$

Equation (5) is an expression for the radius of a bubble as a function of the pressure of the vapor within the bubble.

In order to determine r_o and P_o, we follow the work of Cleary [12], in which the initial pressure P_o at the end of the laser pulse will be taken to be the critical pressure of water of 218 atmospheres. The reasoning behind this is as follows. The kinetics of vaporization is a nonequilibrium process, but eventually equilibrium will be reached between the vapor phase within the bubble and the liquid phase surrounding it. Since there are two distinct phases, the temperature and pressure cannot go above the critical values, which for water are T_c = 374°C and P_c = 218 atmospheres (221 bars), with a critical density of ρ_c = 0.315 g/cm^3 [13]. Since the rate of energy input is faster than either the expansion of the bubble or the heat conduction rate, the critical conditions will at some time be reached if enough energy is absorbed by the melanosome for it to reach 374°C. Therefore, bubble formation can be treated as a process in which the laser energy absorbed by the melanosome creates a saturated vapor with T_o = 374°C and P_o = 218 atmospheres, and whose initial radius r_o is determined by the total energy absorbed by the melanosome. Bubble growth then occurs in an adiabatic expansion that is rapid compared with heat loss. (Note that the maximum volume of the bubble depends on the product $P_oV_o{}^{3/4}$ and that the energy of the laser pulse is used to both vaporize cellular fluid in creating V_o and to elevate the initial pressure P_o. Thus, for a given amount of energy, if the initial nonequilibrium vaporization process does not raise the pressure to a P_o of the full 218 atmospheres, there will be additional energy available to vaporize more cellular fluid, leading to a larger V_o. This tends to limit the variation in the product $P_oV_o{}^{3/4}$ and thus the final volume and radius are not especially sensitive to the actual value used for P_o.)

In order to justify the adiabatic treatment of the bubble growth, the velocity of expansion must be much greater than the rate of heat loss. The characteristics of the expansion can be determined by following the treatment of Lamb [14] for the rate of expansion of the bubble radius:

$$\dot{r}^2 = \frac{2c_o^2}{3(\gamma - 1)}\left[\left(\frac{r_o - a}{r - a}\right)^3 - \left(\frac{r_o - a}{r - a}\right)^{3\gamma}\right] \tag{6}$$

where $c_o = \sqrt{P_o/\rho}$ and ρ is the density of the liquid. The value of r at which the speed of expansion is a maximum is obtained from Equation (6) by taking the derivative of $\dot{r}$ with respect to r, and gives

$$(r - a) = \gamma^{1/(3\gamma - 3)}\,(r_o - a). \tag{7}$$

The maximum rate of expansion that occurs at this value of r is (the following equation is the corrected version of Equation (12), p. 123 of Lamb, and Equation (8) in Cleary [12]):

$$\dot{r}^2_{\max} = \frac{2}{3}c_o^2\gamma^{\gamma/(1-\gamma)}. \tag{8}$$

Finally, the time at which a bubble reaches a radius r during its growth phase can be gotten from Equation (6):

$$t = \frac{r_o - a}{c_o}(2Z)^{1/2}\left(1 + \frac{2}{3}Z + \frac{Z^2}{5}\right), \tag{9}$$

where $Z = (r - r_o)\,/\,(r_o - a)$.

If we insert γ = 4/3 [12,14] into Equation (8), the maximum speed of bubble growth is $\dot{r}_{\max}$ = 0.46 c_o. Using P_o = 218 atmospheres and ρ = 1 g/cm^3 gives $c_o \approx$ 150 m/s and $\dot{r}_{\max} \approx$ 70 m/s,

which is more than two orders of magnitude larger than the characteristic thermal conduction rate of Equation (1). Thus, expansion occurs on a time scale that is much shorter than heat loss, and this justifies the use of an adiabatic treatment during expansion. This can also be seen from Equation (9). If we use representative values of $a = 10^{-6}$m, $r_o = 2a$, the time it takes a bubble to grow to $2r_o$ is on the order of 10^{-7} seconds.

Bubble Size as a Function of Laser Fluence

In studying cellular damage, we are most interested in the maximum size that the bubble reaches, r_m. We now show how the size of a bubble depends on laser fluence and melanosome properties (radius and absorption coefficient). Using Equation (5) to get the maximum bubble size we obtain

$$(r_m/a)^3 = 1 + [(r_o/a)^3 - 1](P_o/P_{min})^{1/\gamma}. \quad (10)$$

The minimum pressure, at which the bubble stops expanding, is taken to be the ambient pressure of 1 atmosphere, and this will be the same for all bubbles. In actuality, the outward momentum of the liquid cellular medium may cause an overshoot in which the bubble's vapor expands to a pressure of less than 1 atmosphere. The effects of this overshoot on V_{max} are mitigated by the factor of $1/\gamma$ in Equation (10). For example, an overshoot in which the pressure drops to ½ atmosphere will result in a final bubble volume that is 69% larger (which corresponds to an r_m that is only 19% larger) than if the pressure goes no lower than 1 atmosphere. Furthermore, this inertial tendency for overshoot tends to be counterbalanced by energy loss during expansion from viscous forces. We therefore use Equation (10) with P_{min} = 1 atmosphere = 1.013×10^5N/m^2.

Using P_o = 218 atmospheres, the ratio P_o/P_{min} is equal to 218 for all laser pulses that have sufficient fluence to raise the melanosome to T_c = 374°C. Therefore, Equation (2) tells us that the maximum volume of the vapor in the bubble is $218^{3/4} = 56.7$ times larger than V_o, with the melanosome occupying a constant volume inside the bubble. This will be true even if the melanosome breaks apart during the process, as long as the pieces remain inside the bubble. To calculate r_m from Equation (10), therefore, requires only an expression for r_o/a, which depends on the energy absorbed by the melanosome.

The energy required to raise 1 gram of water from body temperature of 37°C at 1 atmosphere of pressure to the critical point of 374°C at 218 atmospheres will be denoted by q (the value of q is approximately 2,770 J/g, as shown in Appendix I). At the end of the laser absorption process, the energy E absorbed by a melanosome in the short pulse has created a vaporized volume V_o containing saturated steam and has raised the temperature of the melanosome to the same 374°C. The initial volume of the steam will be

$$V_o = \frac{4\pi}{3}(r_o^3 - a^3) = \frac{E - E_m}{q} \times \frac{1}{\rho_c}, \quad (11)$$

where E_m is the energy required to raise a melanosome from 37°C to 374°C and is equal to $E_m = c_m\rho_m(4\pi/3\ a^3)\ \Delta T$. For $a = 10^{-6}$m, this gives a value of $E_m = 4.8 \times 10^{-9}$ J.

The calculation for r_o continues by evaluating the energy absorbed by the melanosome. For a path length of d through a material with an absorption coefficient α, the fraction of light absorbed is $(1 - e^{-\alpha d})$. If H_o is the fluence of the laser in J/cm^2, then the energy incident on a spherical melanosome is $E_o = \pi a^2 H_o$, where a is the radius of the melanosome. The rigorous expression for the total energy absorbed by a spherical absorber of radius a and absorption coefficient α is derived in Appendix II and is given by

$$E = E_o - E_T = \pi a^2 H_o \times \left(1 - \frac{1}{2\alpha^2 a^2}[1 - e^{-2\alpha a}(1 + 2\alpha a)]\right) = C(\alpha, a)\pi a^2 H_o. \quad (12)$$

Using representative values for α of melanin [15,16] gives $C(\alpha,a)$ for a melanosome of

$$C(1{,}000\ \text{cm}^{-1}, 10^{-6}\text{m}) = 0.124$$
$$C(1{,}800\ \text{cm}^{-1}, 10^{-6}\text{m}) = 0.210$$

$C(\alpha,a)$ can be approximated to second order by $C(\alpha,a) \approx 4/3 \times \alpha a - (\alpha a)^2$, giving for the total energy absorbed,

$$E \approx \left[\frac{4}{3}\alpha a - (\alpha a)^2\right] \pi a^2 H_o. \quad (13)$$

This approximate expression for $C(\alpha,a)$ is accurate to within 1.5% for $\alpha a = .18$ and is accurate to 0.5% for $\alpha a = .10$.

We can use these numbers to get an approximate value for the minimum fluence necessary to produce a bubble in the manner described earlier in which the rapid and intense heating of the melanosome leads initially to a thin shell of vapor close to the critical point. For a 1 μm radius melanosome, we find from Equation (12) or Equation (13) that for $\alpha = 1{,}000\ \mathrm{cm}^{-1}$, the energy absorbed by a melanosome is $E = 3.9 \times 10^{-9}\ \mathrm{cm}^2 \mathrm{H_o}$. Using this in Equation (11) leads to an initial volume that is vaporized of

$$V_o = \frac{4\pi}{3}(r_o^3 - 10^{-12}\mathrm{cm}^3) = \frac{3.9 \times 10^{-9}\mathrm{cm}^2 H_o - 4.8 \times 10^{-9}\mathrm{J}}{q} \times \frac{1}{.315\ \mathrm{g/cm}^3}. \quad (14)$$

Equation (14) represents the scenario in which any energy above $E_m = 4.8 \times 10^{-9}$J absorbed by a 1 μm melanosome is used to produce an initial bubble with the vapor at the critical point. In order for at least 4.8×10^{-9}J to be absorbed, Equation (14) shows that the fluence hitting the melanosome must be at least ($\alpha = 1{,}000\ \mathrm{cm}^{-1}$)

$$H_o^{min} = 4.8 \times 10^{-9}\ \mathrm{J}/3.9 \times 10^{-9}\mathrm{cm}^2 = 1.23\ \mathrm{J/cm}^2. \quad (15a)$$

If the same calculation is done with $\alpha = 1{,}800\ \mathrm{cm}^{-1}$, the energy absorbed becomes $E = 6.6 \times 10^{-9}\ \mathrm{cm}^2 \mathrm{H_o}$ and there is a decrease to

$$H_o^{min} = 0.73\ \mathrm{J/cm}^2 \quad (\alpha = 1{,}800\ \mathrm{cm}^{-1}). \quad (15b)$$

Two comments must be made concerning these $\mathrm{H_o^{min}}$. (1) The value of $\mathrm{H_o^{min}}$ will vary as a function of several factors: wavelength (α), shape, and size (volume of melanosome to be heated depends on a^3 but absorbed energy depends on $C(\alpha,a)$ of Equation (12), which has a complicated dependence on αa). (2) A fluence of less than $\mathrm{H_o^{min}}$ does not mean that no bubble is formed but instead that the initial conditions of the bubble are less extreme than those of a vapor with the critical values of $T_o = 374$°C and $P_o = 218$ atmospheres, and initial bubble formation cannot be treated in the manner leading to Equations (14) and (15). However, the values of $\mathrm{H_o^{min}}$ just calculated are very close to the experimental values for $\mathrm{ED_{50}}$ measurements of a retinal fluence of approximately 1 J/cm^2 for pulse durations less than 10^{-6} seconds. This shows that this treatment of bubble formation and growth is appropriate for analyzing threshold damage, leading to a minimum visible lesion (MVL).

In order to get an expression for the maximum radius attained by the bubble, r_m, we continue with our analysis. Equation (10) shows that r_m/a depends on $[(r_o/a)^3 - 1]$. Using Equation (12) for the energy absorbed and the expression for E_m following Equation (11) (with a given in cm) for the energy needed to raise the temperature of a melanosome from 37°C to 374°C, Equation (11) leads to

$$\left(\frac{r_o}{a}\right)^3 - 1 = \frac{1}{q\rho_c} \times \left[\frac{3C(\alpha a)H_o}{4 \quad a} - c_m\rho_m\Delta T\right]. \quad (16)$$

Using this expression in Equation (10) gives

$$\left(\frac{r_m}{a}\right)^3 = 1 + \frac{1}{q\rho_c} \times \left[\frac{3C(\alpha a)H_o}{4 \quad a} - c_m\rho_m\Delta T\right] \times \left(\frac{\mathrm{P_o}}{\mathrm{P_{min}}}\right)^{1/\gamma}, \quad (17)$$

with a in cm, α in cm^{-1}, and H_o the retinal fluence in J/cm^2. Using the rigorous expression for $C(\alpha,a)$ from Equation (12) gives

$$\left(\frac{r_m}{a}\right)^3 = 1 + \frac{1}{q\rho_c} \times \left[\frac{3}{4a}\left(1 - \frac{1}{2\alpha^2a^2}[1 - e^{-2\alpha a}(1 + 2\alpha a)]\right) H_o - c_m\rho_m\Delta T\right]\left(\frac{P_o}{P_{min}}\right)^{1/\gamma}. \quad (17a)$$

A quicker estimate can be made using the simple expansion for $C(\alpha,a)$ given in Equation (13), which results in

$$\left(\frac{r_m}{a}\right)^3 = 1 + \frac{1}{q\rho_c} \times \left[\left(\alpha - \frac{3}{4}\alpha^2 a\right)H_o - c_m\rho_m\Delta T\right] \times \left(\frac{\mathrm{P_o}}{\mathrm{P_{min}}}\right)^{1/\gamma}. \quad (17b)$$

Comparison of Threshold Fluences (H_o) for Bubble Damage vs. Thermal Damage

By comparing the fluence needed for bubble damage to the fluence needed for thermal dam-

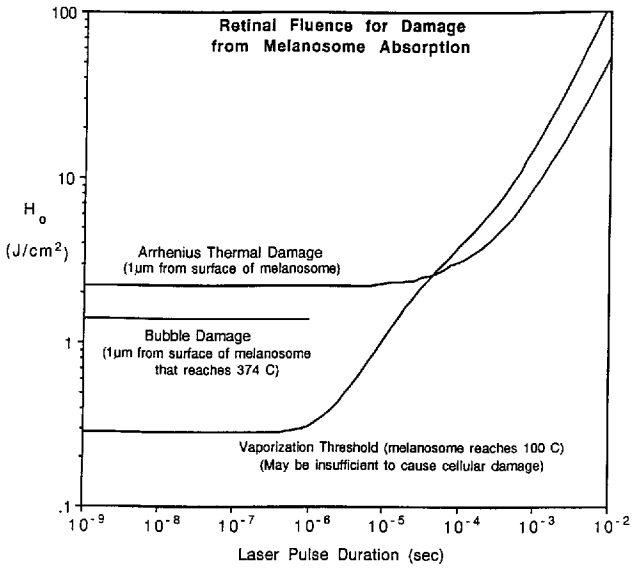

Fig. 1. Threshold retinal fluence necessary for damage caused by different mechanisms. Threshold fluences for bubble damage or Arrhenius thermal damage are calculated for a location that is 1 μm from the surface of a melanosome of 1 μm radius. The absorption coefficient for melanosome is taken to be 1,000 cm^{-1}.

age, we now show that for laser pulse durations in the nanosecond to microsecond range, bubble formation is the mechanism that determines the damage threshold fluence. The values graphed in Figure 1 are retinal fluences. With a focusing power of the eye of approximately 10^5 [17], the equivalent corneal fluences can be obtained by multiplying the retinal fluence by 10^{-5}.

We used the following criteria for calculating and comparing damage fluences. First, we assume that a bubble causes damage only out to distances from the surface of a melanosome that the bubble has actually expanded into. Thus we are underestimating bubble damage by ignoring any damaging effects due to compression and movement of the cellular material outside the actual volume cleared out by the bubble. Next, we assume that cellular damage leading to MVL will occur if primary damage (bubble or thermal) occurs at a distance of 1 μm from the surface of 1 μm melanosome (i.e., r_m = 2 μm from the center of a melanosome).

The choice of this distance is not based on any specific experimental observations but is based on the following conservative reasoning: (1) Assume an RPE cell is 6,000 μm^3 in volume (15 μm × 20 μm × 20 μm). (2) An RPE cell contains approximately 100 melanosomes. (3) The average volume of a melanosome is approximately 4.2 μm^3 (sphere of radius 1 μm) so melanosomes occupy 420 μm^3, which is 7% of the total volume of a cell. (4) If damage (bubble or thermal) occurs out to a radius of 2 μm, then each spherical damage zone now occupies a volume of 33.5 μm^3. (5) One hundred of these damage spheres, if they did not overlap, would occupy 3350 μm^3, which is more than half (56%) of the total volume of the cell. (6) Assume that this damage is enough to kill a cell and thus gives an estimate of the fluence needed for threshold damage.

In Figure 1 we plot three curves. The curve labeled bubble damage is the retinal fluence H_o needed to produce a bubble that expands out 1 μm from the surface of a 1 μm radius melanosome with an absorption coefficient α = 1000 cm^{-1}. The fluence is calculated using the near-critical point bubble formation described earlier, which leads to Equation (16) and gives a fluence of 1.4 J/cm^2. This curve is only plotted in the regime in which it is valid; laser pulses of durations between 10^{-6} and 10^{-9} seconds, and in this regime the required fluence is constant. If α = 1,800 cm^{-1}, this curve drops to H_o = 0.79 J/cm^2.

The curve labelled Arrhenius thermal damage is the retinal fluence needed to produce thermal damage at a distance of 1 μm from the surface of a 1 μm melanosome, again with an absorption coefficient α = 1,000 cm^{-1}. The fluence is calculated by using an Arrhenius expression to model the thermal damage [1–3] in which damage occurs when

$$\sum C_1 e^{\frac{-C_2}{310K + \Delta T(t)}} \Delta t = 1. \qquad (18)$$

The values for C_1 and C_2 were taken from Takata [2]:

$C_1 = 4.322 \times 10^{64}$/sec $\quad T \leq 323$ K
$C_2 = 50{,}000$ K

$C_1 = 9.389 \times 10^{104}$/sec $\quad T > 323$ K
$C_2 = 80{,}000$ K

The fluence necessary to cause thermal damage is determined by Equation (18) since it is the fluence that determines the time dependence of ΔT. The details of the computations will be published in a separate paper [18].

It is important to note that for thermal damage, we looked at a point that is 1 μm from the surface of a specific melanosome, but that 99

other melanosomes were randomly placed by the computer code in a cellular volume of 6,000 μm^3. Thus, as expected in actual experiments, the location examined also received heat from other melanosomes that were further away than 1 μm, but could still be relatively nearby. The result for this computation was that for pulses of duration shorter than 10^{-6} seconds, 2.1 J/cm^2 were needed to cause thermal damage, noticeably higher than the 1.4 J/cm^2 calculated for bubble damage.

The third curve in Figure 1, labelled the vaporization threshold, shows the retinal fluence needed [18] to raise the temperature of a melanosome to 100°C, at which point bubble formation will begin, though not with the near critical point conditions discussed in this paper. This fluence of approximately 0.29 J/cm^2 is well below measured threshold ED_{50}'s for minimal spot sizes (see the following) and therefore is evidently insufficient for causing MVL. This curve does have the value, however, of showing how heat conduction away from a melanosome plays an important role for time scales greater than 10^{-6} seconds. Thus for laser pulses of duration greater than a microsecond, significant energy conducts away from the melanosome into the cellular media during the time in which energy is being deposited. This is why thermal damage, which requires smaller temperature rises that last for extended times, becomes easier to cause (smaller H_0) than bubble formation for pulse durations greater than 10^{-5} seconds.

Finally, we note that for a given pulse duration there is experimental evidence of an inverse relationship between damage threshold fluences and retinal image size. As seen in Sliney and Wolbarsht [19], this holds over a wide range of pulse durations. The present model does not include any threshold dependence on retinal image size since even the smallest image size of approximately 20 μm will encompass hundreds of melanosomes and the damage mechanism is due to bubbles forming around each melanosome independently of the others. Thus, in this model the same fluence (J/cm^2) will cause the same size bubble around a melanosome whether the image size is 20 μm or 1,000 μm. Therefore, for the same fluence the same amount of damage will occur around each individual melanosome within the image, the difference being that a larger image size will cause the same damage per unit area (density of damage) but over a much larger area.

A possible explanation for the decrease in threshold fluence with increasing spot size is that, since these threshold levels are determined ophthalmoscopically, it may be that for large spot sizes damage is noticeable at lower levels of damage per unit area than for smaller spot sizes. In terms of the bubble damage model of this paper, this implies that for larger image sizes the bubbles need not grow as large to cause ophthalmoscopically visible damage and thus the required threshold fluence levels will decrease as the spot size increases, as observed experimentally by Sliney and Wolbarsht [19]. We emphasize that this explanation is only speculative concerning the interesting relationship between threshold fluence and spot size.

RESULTS

Equations (17) show explicitly how r_m depends on the pulse fluence H_0 and on melanosome properties α, a, and q. We first get a representative value for r_m by using representative values for all the parameters in Equation (17a) as listed earlier in this report: $a = 10^{-6}$ $m = 10^{-4}$ cm, $c_m = 2.51$ J/g·°C, $\rho_m = 1.35$ g/cm^3, $\Delta T = 374°C - 37°C = 337°C$, $\rho_c = 0.315$ g/cm^3, $P_0 = 218$ atmospheres, $P_{min} = 1$ atmosphere, $\gamma = 4/3$, and $q = 2{,}770$ J/q. For example, for $H_0 = 1.5$ J/cm^2, Equation (17a) gives the following results for two different values of α:

$$\alpha = 1{,}000\ cm^{-1} \rightarrow r_m = 2.6\ \mu m,$$
$$\alpha = 1{,}800\ cm^{-1} \rightarrow r_m = 4.3\ \mu m.$$

The following tables show how the maximum bubble radius, r_m, varies as a function of different parameters. Each table shows the variation of r_m with the melanosome properties α and a, for various laser fluences H_0. Entries of 0.0 do not mean that no bubble is formed but instead signify that the present model is not applicable because not enough energy was absorbed by the melanosome to raise the temperature of the melanosome to $T_c = 374°C$.

DISCUSSION

The calculation leading to the results of Equation (15) is strong support for both the validity of the model used in this report and the importance of bubble formation in causing minimal visible lesions for short laser pulses (10^{-6} to 10^{-9} seconds) incident on the eye. Our calculations show that bubble damage will occur for a retinal

fluence of 1.4 J/cm^2 for an "average" melanosome with a radius of 1 μm and α = 1,000 cm^{-1}, or only 0.79 J/cm^2 if α = 1,800 cm^{-1}. These calculated H_o^{min}'s agree well with the experimental ED_{50}'s measured for short pulses, which are found to be approximately 1 J/cm^2. This close agreement supports the idea that bubble formation is a cause of MVL in short pulses and that this model is a reasonable theoretical treatment for calculating the size of damage-causing bubbles as a function of the relevant parameters.

The importance of various parameters for bubble growth can be ascertained from Equation (17) and the information in Table 1: (1) The dependence of r_m on the ratio of the pressures at the beginning and end of the bubble expansion is $(P_o/P_{min})^{-1/3\gamma}$. With $\gamma = 4/3$, this gives a weak dependence on $(P_o/P_{min})^{-1/4}$. Thus a major change in the ratio of P_o/P_{min}, such as by a factor of two, leads to a change in V_{max} by a factor of 1.69 (corresponding to a change in r_m by a factor of only 1.19). (2) The dependence of r_m on the melanosome radius a, absorptivity α, and the laser retinal fluence H_o is complex due to the nonlinear way in which these parameters influence r_m. Thus, how strong a dependence r_m has on any one of these depends on the specific values of the other two.

A few general trends are discernible: (1) r_m increases monotonically with α and H_o, but not linearly, as seen in Table 1 and plotted in Figure 2a and 2b. The dependence is better categorized as a threshold dependence, as expected from Equation (17). (2) Because of its appearance in several terms in Equation (17a), the dependence on a is much more complicated and not always monotonic. Even the ratio r_m/a is not monotonic, and for certain values of α and H_o, increasing a leads to decreasing r_m. This occurs around threshold values for H_o; see the columns with α = 1,800 cm^{-1} in H_o = 0.75 and 1,400 cm^{-1} in H_o = 1.00.

This paper presents a theoretical approach for calculating the maximum bubble size formed in retinal pigment epithelium cells due to short laser pulses with pulse durations in the range of 10^{-6} to 10^{-9} seconds. The agreement between the threshold energy for the formation of bubbles calculated by the model with the experimental ED_{50} shows the relevance of the model for damage assessment. A full understanding of the damage process requires additional work directed towards understanding the mechanisms by which bubbles actually cause cellular damage. These mechanisms will obviously derive from the physical expansion of the bubble and the manner in which

TABLE 1. Maximum Bubble Radius r_m, Calculated from Equation (17a), as a Function of Melanosome Radius a, Melanosome Absorptivity α, and Laser Retinal Fluence H_o

	α(cm^{-1})			
a(μm)	600	1,000	1,400	1,800
H_o(J/cm^2): 0.50		r_m		
0.50	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00
1.25	0.00	0.00	0.00	0.00
1.50	0.00	0.00	0.00	0.00
1.75	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00
H_o(J/cm^2): 0.75				
0.50	0.00	0.00	0.00	1.04
0.75	0.00	0.00	0.00	1.38
1.00	0.00	0.00	0.00	1.55
1.25	0.00	0.00	0.00	1.38
1.50	0.00	0.00	0.00	0.00
1.75	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00
H_o(J/cm^2): 1.00				
0.50	0.00	0.00	1.18	1.65
0.75	0.00	0.00	1.67	2.40
1.00	0.00	0.00	2.07	3.09
1.25	0.00	0.00	2.38	3.71
1.50	0.00	0.00	2.55	4.28
1.75	0.00	0.00	2.53	4.77
2.00	0.00	0.00	2.13	5.19
H_o(J/cm^2): 1.25				
0.50	0.00	0.86	1.63	2.00
0.75	0.00	1.15	2.38	2.92
1.00	0.00	1.31	3.09	3.80
1.25	0.00	0.00	3.75	4.64
1.50	0.00	0.00	4.35	5.42
1.75	0.00	0.0	4.91	6.16
2.00	0.00	0.00	5.41	6.85
H_o(J/cm^2): 1.50				
0.50	0.00	1.37	1.92	2.25
0.75	0.00	2.00	2.82	3.31
1.00	0.00	2.59	3.68	4.32
1.25	0.00	3.13	4.51	5.29
1.50	0.00	3.63	5.30	6.22
1.75	0.00	4.07	6.04	7.11
2.00	0.00	4.46	6.75	7.95
H_o(J/cm^2): 2.00				
0.50	0.71	1.87	2.32	2.63
0.75	0.97	2.76	3.43	3.88
1.00	1.11	3.62	4.50	5.09
1.25	0.00	4.46	5.54	6.26
1.50	0.00	5.27	6.55	7.39
1.75	0.00	6.04	7.52	8.48
2.00	0.00	6.79	8.46	9.53
H_o(J/cm^2): 3.00				
0.50	1.72	2.43	2.85	3.17
0.75	2.56	3.61	4.23	4.69
1.00	3.38	4.76	5.57	6.17
1.25	4.18	5.89	6.89	7.60
1.50	4.96	7.00	8.16	9.00
1.75	5.73	8.08	9.41	10.36
2.00	6.47	9.14	10.63	11.67

Other parameters are as follows: c_m = 2.51 J/g·°C, ρ_m = 1.35 g/cm^3, ΔT = 337°C, ρ_c = 0.315 g/cm$_3$, P_o/P_{min} = 218, γ = 4/3, and q = 2770 J/g.

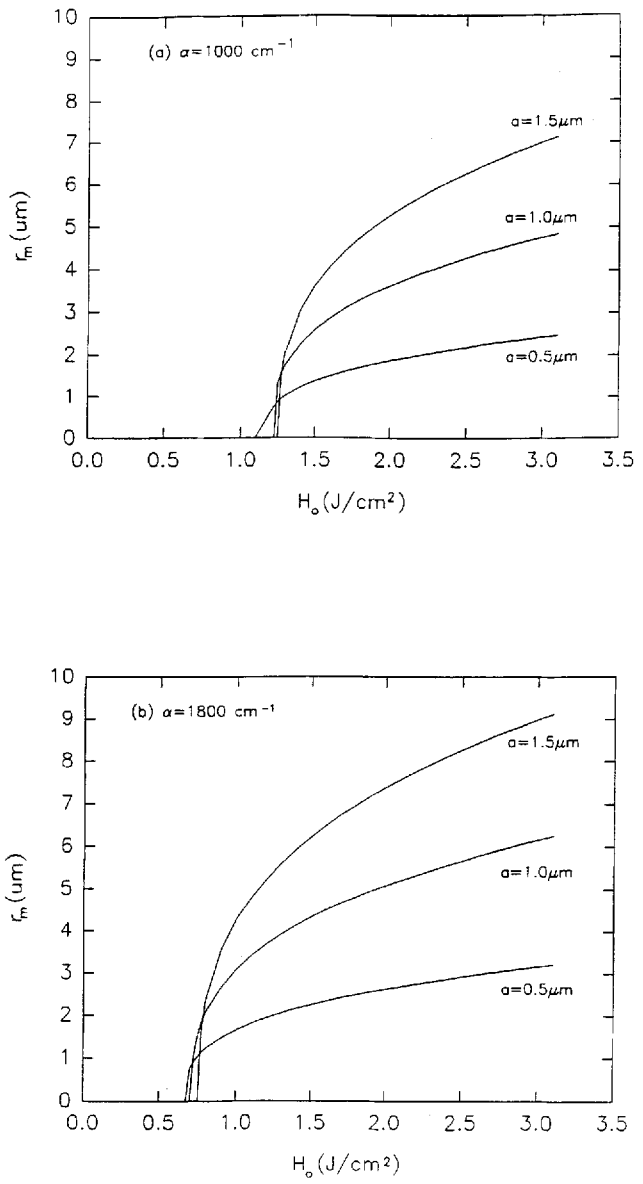

Fig. 2. Maximum bubble radius as a function of retinal fluence, showing a threshold dependence. **a** and **b** differ in the absorption coefficient used for the melanosome.

the physical expansion destructively couples to the functioning of a cell. Does the expansion destroy enough cellular proteins to cause MVL immediately? Or is the damage initially minor but enough to prevent the cell's biochemical pathways from repairing the initially damaged areas, as well as disrupting transport channels sufficiently so that the cell is not able to continue carrying out its usual functions at the necessary rates?

In order to distinguish between mechanisms such as these and others, experiments must be done that look at immediate effects of the laser pulse. Immediate effects, in this case, are as soon as the bubble has finished expansion, which is on the submillisecond time scale. This requires use of experimental methods such as pump-probe optical techniques that look for changes in protein absorption characteristics on these time scales.

Finally, we note that another paper [18] reports on a more accurate computational method for predicting temperature rises produced by relatively long laser pulses ($\tau > 10^{-5}$ seconds) for which heat is conducted away fast enough so that ED_{50}'s imply no vaporization. For laser pulses of duration in the range of 10^{-5} to 10^{-6} seconds, the theoretical treatment will be complicated by the presence of both vaporization and conduction, and remains to be investigated in detail. Also, for pulse durations under a nanosecond, the damage analysis of this paper may not be valid due to shock wave formation. In this subnanosecond pulse length regime of "stress confinement," mechanical waves created by the laser do not have time to leave the melanosome during the duration of the laser pulse and a significant fraction of the absorbed energy can be used in generating mechanical stress and shock waves, rather than bubble formation. This may be responsible for the apparent lowering of the ED_{50} threshold for damage for laser pulses shorter than 10^{-10} seconds.

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APPENDIX I

Calculation of *q*

The value for q, the number of joules of energy required to raise 1 gram of water from 37°C at 1 atmosphere of pressure to the critical point at 374°C and 218 atmospheres, is determined from thermodynamic considerations:

$$\Delta E = \Delta H - \Delta(PV), \qquad \text{(A1)}$$

where the energy change q is represented in Equation (A1) by E, and H is the enthalpy of the process. Since the change in energy is a state function, we can use any path in $P - T$ space to evaluate ΔE. We use the path shown in Figure A1 in which, first, at a constant pressure of 1 atmosphere, water is raised from body temperature to the critical temperature. Since $dH = dQ + VdP$, during this constant pressure part of the process $\Delta H = dQ$ even though volume changes do occur. The heat required to raise water from a body temperature of 37°C to 100°C is 263 J/g. The water is then transformed to vapor at 1 atmosphere pressure, which requires heat of 2,262 J/g. Heat is then used to raise the temperature of the steam from 100°C (373°K) to the critical temperature of 374°C (647°K). The heat capacity for steam at a constant pressure of 1 atmosphere increases over this temperature range, and we use the following expression for c_p [20]:

$$c_p = a + (b \times 10^{-3})T + (c \times 10^{-6})T^2, \qquad \text{(A2)}$$

with $a = 1.67$ J/g·°K, $b = 0.59$ J/g·°K^2, and $c = 0.019$ J/g·°K^3. Performing the integral of $\int c_p dT$ from 373°K to 647°K gives a heat of 541 J/g. Thus, the enthalpy change in raising the temperature of H_2O from 37°C to 374°C, all at 1 atmosphere of pressure, is 3,066 J/g.

The remaining changes in energy needed for evaluating Equation (A1) are ΔH due to the step in which the temperature remains constant at 647°K and the pressure is increased isothermally from 1 atmosphere to 218 atmospheres, as well as the evaluation of $\Delta(PV)$ in Equation (A1) for the entire process. Since the product PV is also a state function, we can ignore intermediate steps and evaluate $\Delta(PV) = P_cV_C - P_oV_o$ with $P_c = 218$ atmospheres $= 221 \times 10^5$ N/m^2, $V_c = 3.17$ cm^3/g, $P_o = 1$ atmosphere $= 1.013 \times 10^5$ N/m^2, and $V_o = 1$ cm^3/g. This gives $\Delta(PV) = 70$ J/g. The ΔH for the isothermal compression of the steam at 647°C from $P_o = 1$ atmosphere to $P_c = 218$ atmospheres can be evaluated by rewriting Equation (A1) as $\Delta H = \Delta E + \Delta(PV)$. If the steam behaved as an ideal gas during the isothermal compression, then we would have $\Delta H = 0$ since $E = E(T)$ for an ideal gas and $PV = RT$, so $\Delta E = 0$ and $\Delta(PV) = 0$.

In actuality the steam may behave as an ideal gas at $P_i = 1$ atmosphere but does not behave as an ideal gas as the critical pressure is reached. Treating steam as an ideal gas at 1 atmosphere and using $\rho_c = .315$ g/cm^3 allows the evaluation of $\Delta(PV) = P_cV_c - P_iV_i$ for this step: $P_cV_c = 70$ J/g, and P_iV_i (ideal) $= RT = 299$ J/g. This gives $\Delta(PV) = -230$ J/g. The evaluation of

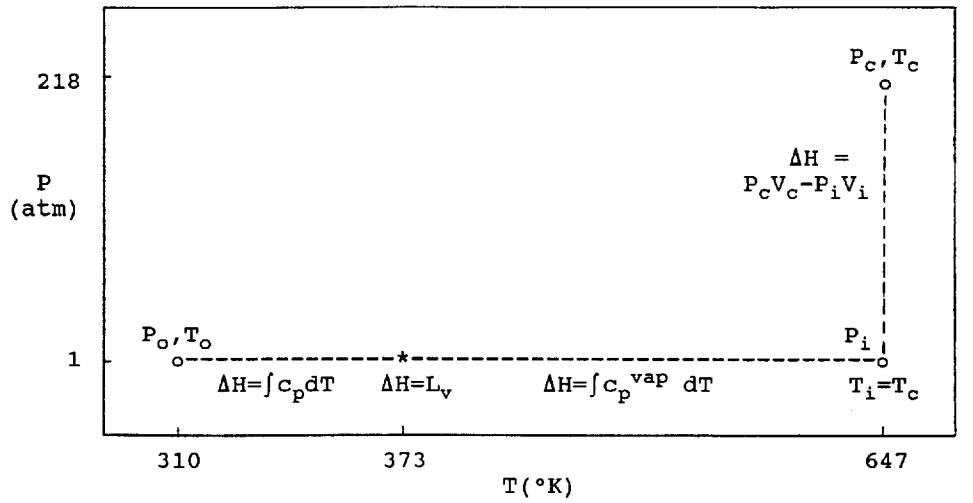

Fig. A1. Evaluation of enthalpy changes, ΔH, in determination of $q = \Delta E = \Delta H - \Delta(PV)$. In addition to ΔH, there is the overall $\Delta(PV) = P_cV_c - P_oV_o$. An expression for c_p^{vap} is given in Equation (A2).

ΔE is not as straightforward. During this isothermal compression process, work is done on the steam, which tends to increase its energy. However, in order for the temperature to remain constant, this energy must be either lost to heat ΔH or used in bond modifications. If the steam behaved as an ideal gas, then its internal energy, which is purely kinetic for an ideal gas, would not change and this $\Delta E = 0$ would imply $\Delta H = \Delta(PV)$. In a non-ideal gas, however, there are bonds between molecules and it is possible to increase the energy of the system without a corresponding temperature increase. Thus, in compressing the steam isothermally, some of the energy put in as work can stay in the system and need not be lost as heat. Nevertheless, since we are compressing a vapor in which the interactions between molecules are much weaker than in liquids, and since a phase transformation does not occur during the compression, we will assume that the change in internal energy is small since T remains constant and set $\Delta E = 0$ for this isothermal compression. The actual value could be determined from the virial coefficients of steam under the appropriate conditions of temperature and pressure. With $\Delta E = 0$, we have $\Delta H = \Delta(PV) = -230$ J/g for the isothermal compression.

Adding up all the contributions to ΔH we get $\Delta H = 3066$ J/g $- 230$ J/g $= 2836$ J/g. The overall $\Delta(PV) = 70$ J/g. Inserting these numbers into Equation (A1) gives $q = \Delta E \approx 2{,}770$ J/g for use in Equation (17), with an uncertainty due to setting $\Delta E = 0$ for the isothermal compression, as explained in the previous paragraph.

APPENDIX II

Energy Absorption by a Spherical Absorber

For light of uniform fluence H_0 (J/cm^2) hitting a sphere of radius a with uniform absorption coefficient α, the fraction of light absorbed is calculated as follows. The total energy hitting the sphere is $E_o = \pi a^2 H_o$. For a path length of d, the fraction of light that passes through is $e^{-\alpha d}$. The average of this fraction over a sphere gives the energy E_T that is transmitted:

$$\frac{E_T}{E_o} = \frac{1}{\pi a^2} \int_o^a e^{-2\alpha\sqrt{a^2-r^2}} 2\pi r dr, \qquad \text{(A3)}$$

where r for a light ray is the distance of closest approach to the center, and $2\sqrt{a^2-r^2}$ is the path length of the light ray through the melanosome. Figure A2 is a diagram of the process. The integration can be performed with a change of variable of $u = 2\alpha\sqrt{a^2-r^2}$ and gives

$$\frac{E_T}{E_o} = \frac{1}{2\alpha^2 a^2}[1 - e^{-2\alpha a}(1 + 2\alpha a)]. \qquad \text{(A4)}$$

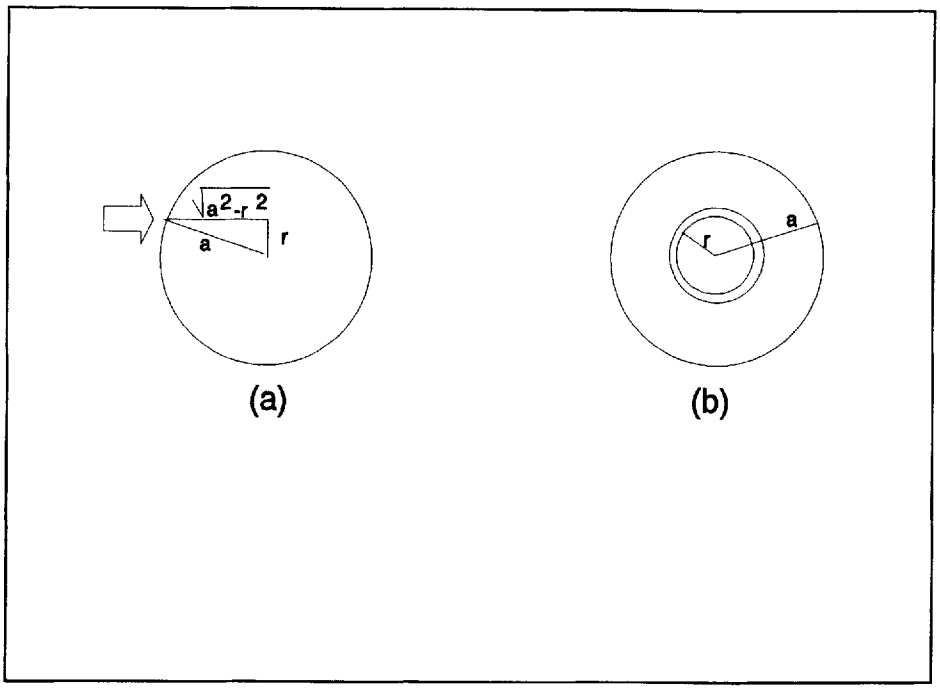

Fig. A2. Absorption by spherical melanosome. (**a**): Side view showing distance of closest approach, *r*, of light from center of melanosome. (**b**): Front view of melanosome as seen by approaching light.

The energy absorbed is

$$E = E_o - E_T$$
$$= \pi a^2 H_o \left[1 - \frac{1}{2\alpha^2 a^2}(1 - e^{-2\alpha a}(1 + 2\alpha a)) \right]$$
$$= C(\alpha,a)\pi a^2 H_o. \quad \text{(A.5)}$$

For a sphere that has the properties of a melanosome with $a = 1$ μm and a visible light $\alpha = 1{,}000$ cm^{-1}, the fraction of light absorbed $C(\alpha,a)$ is 0.124 and $E = 0.124E_o = 0.124\ (\pi a^2 H_o)$. For $\alpha = 1{,}800$ cm^{-1}, $C(\alpha,a) = 0.210$.